

# The Long Road of Immunotherapeutics against Multiple Sclerosis <sup>†</sup>

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<sup>†</sup> This Editorial is dedicated to Elizabeth Matsoukas who was the inspiration of the research.

Received: 24 April 2020; Accepted: 26 April 2020; Published: 11 May 2020

**Abstract:** This commentary highlights novel immunomodulation and vaccine-based research against multiple sclerosis (MS) and reveals the amazing story that triggered this cutting-edge MS research in Greece and worldwide. It further reveals the interest and solid support of some of the world's leading scientists, including sixteen Nobel Laureates who requested from European leadership to take action in supporting Greece and its universities in the biggest ever financial crisis the country has encountered in the last decades. This support endorsed vaccine-based research on MS, initiated in Greece and Australia, leading to a worldwide network aiming to treat or manage disease outcomes. Initiatives by bright and determined researchers can result in frontiers science. We shed light on a unique story behind great research on MS which is a step forward in our efforts to develop effective treatments for MS.

**Keywords:** multiple sclerosis; MS; vaccine; immunomodulation; carriers

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## 1. Introduction

### *Nobel Laureates Taking Action to Support Research in Greece*

It was realized and clearly understood by the governments in Greece five years ago, and especially now, during this period of COVID-19 pandemic, the necessity for research, as first priority in their policies, for innovation, development and growth. Greece has suffered a lot the last decade from recession. Initiatives by eminent scientists were taken to support research in Greece, with remarkable positive outcomes. Fifteen Nobel Laureates cosigned the “Support for Greece” petition that was addressed by Nobel Laureate Professor Harald zur Hausen, on 14 December 2015, to the European leadership (Jean-Claude Juncker, Martin Schulz and Donald Tusk), pleading for the support for research in universities and to the country. The first to sign was the DNA discoverer Nobel Laureate Professor James Watson, who also sent a letter to the then President of the USA, Mr. Barack Obama, urging him to support Greece [1]. This petition to support research and universities in Greece led to the Hellenic Foundation for Research and Innovation (HFRI) to spur economic development. The European Investment Bank co-financed the creation of the HFRI fund with the Ministry of Finance. Professor Kostas Fotakis, Alternate Minister of Research and Technology then, has greatly contributed to the establishment of HFRI. The HFRI fund launches regular calls for all scientists at all stages in support of their research. The “Support for Greece” petition, which was co-signed by the Nobel Laureates and led to the HFRI fund, was a joint initiative between Professor John Matsoukas from the University of Patras Greece and Nobel Laureate Professor Harald zur

Hausen from the German Cancer Research Center in Heidelberg, Germany. This is the second petition after the first in 2012 co-signed by twenty-one Nobel Laureates and addressed again by Professor Harald zur Hausen [2,3]. The second petition worked out successfully.

## 2. The Sparkle of Immunotherapeutics MS Research

Nobel Laureates Professors James Watson (Cold Spring Harbor Laboratory, New York, NY, USA), Harald zur Hausen (German Cancer Research Center, Heidelberg, Germany) and Andrew Schally (University of Miami, FL, USA) were attracted by the excellent research in Greece and have stated in particular that MS research in Greece is world-class research that is worthy of support. This research had its reason and sparkle. Myelin based immunotherapeutics research for MS in Greece was triggered by Dr. Elizabeth Matsoukas, a Biologist, who has been struck by the disease. That happened to her in 1982, at the age of 30. Following her diagnosis, she dedicated her life to promote research for MS. Her PhD dissertation from the National Hellenic Research Foundation in Athens identified and evaluated myelin epitopes, in particular myelin basic protein (MBP) epitopes, which are implicated in the pathogenesis of the disease [4–7]. Now these epitopes are the tools and the core for developing therapeutics and vaccines for the treatment of MS.

## 3. The First EAE Experiment in Pennsylvania

In 1994, Professor John Matsoukas, brother of Elizabeth, decided to introduce into his drug discovery research program at the University of Patras the design, synthesis and development of drugs, mimetics and immunotherapeutics, using MBP epitopes against MS. The first experiment, “experimental allergic encephalomyelitis” (EAE), an animal model of the disease, was run that year, at the University of Pennsylvania, in Professor Abdolmohamad Rostami’s lab, at that time professor of neurology at the University of Pennsylvania (currently Professor and Chairman of the Department of Neurology at Thomas Jefferson University, Philadelphia USA) [8–10]. Elizabeth had visited Professor Rostami earlier that year in his Pennsylvania clinic for diagnosis and prescription of an interferon drug for her case which was not possible yet at that time in Europe. First experiments were carried out, using the guinea pig epitope MBP<sub>72–85</sub>, as suggested by Elizabeth [10]. The research for new therapies was part of her curiosity to determine the mechanisms of disease and, based on that, to pursue treatment of disease. Her first EAE experiments in Pennsylvania were successful and paved the way for further research to identify new peptide immunomodulators, which resulted in research based on the other myelin epitopes, primarily MPB<sub>83–99</sub>, MOG<sub>35–55</sub> and PLP<sub>139–151</sub> [4–7,11–21]. This research was quickly spread to the research community in the field, all over the world.

## 4. Development of a Worldwide MS Consortium

A multi-institutional and multidisciplinary consortium was established in 1999, by Professor John Matsoukas (from the University of Patras; currently Head of NewDrug P.C, Patras Science Park, Greece) and Professor Vasso Apostolopoulos (from the Austin Research Institute Australia/Scripps Research Institute USA); currently Pro Vice-Chancellor, Research Partnerships at Victoria University Australia). The consortium comprised over 15 top universities and research Institutions worldwide (Europe, USA, Canada and Australia), and over 50 researchers have taken part in the consortium over time. Consortium members/collaborators were included, as each had expertise in various disciplines including, chemistry, structural biology, crystallography, molecular dynamics, nuclear magnetic resonance, protein chemistry, cell biology, biochemistry, molecular biology, immunology, neuropathology, animal research, clinical research and neurology clinicians. Each team approached the MS immunotherapeutics research program, using their specialist discipline areas, which together resulted in novel findings and potential new immunotherapeutics against MS. In addition, Professor John Matsoukas (organic chemist) and his team pioneered, through rational design, cyclic constraints of myelin peptides of architectural beauty, which were evaluated for efficacy and stability by members of the consortium. In addition, his team developed

novel altered peptide ligands of myelin peptides. The linear and cyclic peptides, native or as altered peptide ligands, were evaluated for stability in vitro, binding affinities to major histocompatibility complex class II, efficacy in mice and rats and to human peripheral blood mononuclear cells from patients with MS by various consortium groups [6,12–14,22–28].

### 5. Optimizing Immunotherapeutics and Vaccines against MS, Using a Novel Delivery System

Professor Vasso Apostolopoulos (immunologist and crystallographer), who had developed a novel antigen delivery system against breast and ovarian cancer [29–43], which were translated into human clinical trials [44–48], applied her insights into MS research [26,49–54]. The delivery system specifically targets dendritic cells and, when applied to myelin peptides (cyclic, linear and altered peptide ligands), was able to modulate immune responses from pro-inflammatory to anti-inflammatory, with protection and reversal of EAE in animal models and altered cytokine profile in peripheral blood mononuclear cells isolated from patients with MS [26,49–54]. Over 10 candidate immunotherapeutics have been developed and are justified for their use in phase I human clinical trials.

### 6. Awarding the Inspiration and the Pioneers of this MS Research

Dr. Elizabeth Matsoukas can justifiably be proud of what she has achieved. Her pain was translated into promising global research to fight the disease. Her dream to see novel immunotherapeutics development against the disease is very close to being materialized. At last, she has seen research due to her case flourish globally. Elizabeth was honored by the Greek Academy of Athens for her dissertation and in 2018, by His Excellency, the President of the Hellenic Democracy, Mr. Prokopis Pavlopoulos, for her initial research and for being the inspiration, the spur and the motivating power of this research. In a special ceremony on 22 September 2018, in Amaliada (province of Ilida), celebrating 20 years of Medicinal Chemistry excellence in Greece, she was awarded by the president with a DNA-inspired plaque made by famous sculptor Eustathios Leontis. Standing-ovation applause for her contribution was an emotional moment. In this special ceremony, the protagonists of this MS research, Professors Apostolopoulos, Rostami and Matsoukas, were also awarded, as well Professor Harald zur Hausen, for his contribution to science and society. In addition, Professor Vasso Apostolopoulos and Nobel Laureate Professor Harald zur Hausen received an award for career excellence by His Excellency, the President of the Hellenic Democracy, Mr. Prokopis Pavlopoulos. Last year, Professor John Matsoukas and Professor Vasso Apostolopoulos, were each independently awarded the Salus Index Award, from New Times Publishing, for outstanding career achievements, including their work on MS.

### 7. Conclusions

The development of drugs, immunotherapeutics and vaccines against diseases is a long process, often taking researchers a lifetime. Researchers often work in silos, limiting their research output; as such, the breaking down of silos would improve research outcomes. Here, we provided an insight of a multi-institutional and multidisciplinary consortium which was developed over 20 years ago that has led to the identification and development of over 10 candidate immunotherapeutics against MS. Today, most research-funding bodies, require multi-institutional and multidisciplinary teams in order to be successful in grant applications. Most importantly, alliances are required to get to the target of the research.

**Author Contributions:** Conceptualization, J.M. and V.A.; writing, review and editing, J.M., A.R. and V.A. All authors have read and agreed to the published version of the manuscript.

**Funding:** The writing of this commentary received no external funding.

**Acknowledgments:** V.A. would like to thank the Institute for Health and Sport, Victoria University, for supporting her current efforts into MS research. J.M. would like to thank the General Secretariat for Research and Technology (GSRT) for supporting his MS research.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Nobel Laureates “Support for Greece”. Available online <http://www.elenapanaritis.com/22-nobelprize-winners-publish-support-greece-letter-science/> (accessed on 4 May 2020).
2. Syriza may have lost the election, but Greece’s research reforms deserve to stay. Available online: <https://www.nature.com/articles/d41586-019-02323-y> (accessed on 4 May 2020).
3. Hausen, H.Z. Support for Greece. *Sci.* **2012**, *336*, 978–979, doi:10.1126/science.336.6084.978.
4. Tselios, T.; Daliani, I.; Deraos, S.; Thymianou, S.; Matsoukas, E.; Troganis, A.; Gerothanassis, I.; Mouzaki, A.; Mavromoustakos, T.; Probert, L.; et al. Treatment of experimental allergic encephalomyelitis (EAE) by a rationally designed cyclic analogue of myelin basic protein (MBP) epitope 72–85. *Bioorganic Med. Chem. Lett.* **2000**, *10*, 2713–2717, doi:10.1016/S0960-894X(00)00556-4.
5. Tselios, T.; Daliani, I.; Probert, L.; Deraos, S.; Matsoukas, E.; Roy, S.; Pires, J.; Moore, G.; Matsoukas, J. Treatment of experimental allergic encephalomyelitis (EAE) induced by guinea pig myelin basic protein epitope 72–85 with a Human MBP 87–99 analogue and effects of cyclic peptides. *Bioorganic Med. Chem.* **2000**, *8*, 1903–1909, doi:10.1016/S0968-0896(00)00134-6.
6. Tselios, T.; Probert, L.; Daliani, I.; Matsoukas, E.; Troganis, A.; Gerothanassis, I.P.; Mavromoustakos, T.; Moore, G.J.; Matsoukas, J.M. Design and Synthesis of a Potent Cyclic Analogue of the Myelin Basic Protein Epitope MBP72-85: Importance of the Ala81Carboxyl Group and of a Cyclic Conformation for Induction of Experimental Allergic Encephalomyelitis. *J. Med. Chem.* **1999**, *42*, 1170–1177, doi:10.1021/jm980250e.
7. Tselios, T.; Probert, L.; Kollias, G.; Matsoukas, E.; Roumelioti, P.; Alexopoulos, K.; Moore, G.; Matsoukas, J. Design and synthesis of small semi-mimetic peptides with immunomodulatory activity based on Myelin Basic Protein (MBP). *Amino Acids* **1998**, *14*, 333–341, doi:10.1007/bf01318852.
8. Rostami, A.; Gregorian, S.K. Peptide 53–78 of myelin P2 protein is a T cell epitope for the induction of experimental autoimmune neuritis. *Cell. Immunol.* **1991**, *132*, 433–441, doi:10.1016/0008-8749(91)90040-i.
9. Rostami, A.; Gregorian, S.K.; Brown, M.J.; Pleasure, D.E. Induction of severe experimental autoimmune neuritis with a synthetic peptide corresponding to the 53–78 amino acid sequence of the myelin P2 protein. *J. Neuroimmunol.* **1990**, *30*, 145–151, doi:10.1016/0165-5728(90)90098-8.
10. Tselios, T.; Deraos, S.; Matsoukas, E.; Panagiotopoulos, D.; Matsoukas, J.; Moore, G.J.; Probert, L.; Kollias, G.; Hilliard, B.; Rostami, A.; et al. Myelin Basic Protein Peptides: Induction and Inhibition of Experimental Allergic Encephalomyelitis. *Rev. Clin. Pharmacol. Pharmacokinetics* **1997**, *11*, 60–64.
11. Dargahi, N.; Katsara, M.; Tselios, T.; Androutsou, M.-E.; De Courten, M.P.J.; Matsoukas, J.; Apostolopoulos, V. Multiple Sclerosis: Immunopathology and Treatment Update. *Brain Sci.* **2017**, *7*, 78, doi:10.3390/brainsci7070078.
12. Day, S.; Tselios, T.; Androutsou, M.-E.; Tapeinou, A.; Frilligou, I.; Stojanovska, L.; Matsoukas, J.; Apostolopoulos, V. Mannosylated Linear and Cyclic Single Amino Acid Mutant Peptides Using a Small 10 Amino Acid Linker Constitute Promising Candidates Against Multiple Sclerosis. *Front. Immunol.* **2015**, *6*, doi:10.3389/fimmu.2015.00136.
13. Deraos, G.; Kritsi, E.; Matsoukas, M.-T.; Christopoulou, K.; Kalbacher, H.; Zoumpoulakis, P.; Apostolopoulos, V.; Matsoukas, J. Design of Linear and Cyclic Mutant Analogues of Dirucotide Peptide (MBP82–98) against Multiple Sclerosis: Conformational and Binding Studies to MHC Class II. *Brain Sci.* **2018**, *8*, 213, doi:10.3390/brainsci8120213.
14. Deraos, G.; Rodi, M.; Kalbacher, H.; Chatzantoni, K.; Karagiannis, F.; Synodinos, L.; Plotas, P.; Papalois, A.; Dimisianos, N.; Papathanasopoulos, P.; et al. Properties of myelin altered peptide ligand cyclo(87-99)(Ala91,Ala96)MBP87-99 render it a promising drug lead for immunotherapy of multiple sclerosis. *Eur. J. Med. Chem.* **2015**, *101*, 13–23, doi:10.1016/j.ejmech.2015.06.015.
15. Laimou, D.; Lazoura, E.; Troganis, A.N.; Matsoukas, M.-T.; Deraos, S.N.; Katsara, M.; Matsoukas, J.; Apostolopoulos, V.; Tselios, T. Conformational studies of immunodominant myelin basic protein 1–11 analogues using NMR and molecular modeling. *J. Comput. Mol. Des.* **2011**, *25*, 1019–1032, doi:10.1007/s10822-011-9481-6.
16. Matsoukas, J.; Apostolopoulos, V.; Kalbacher, H.; Papini, A.M.; Tselios, T.; Chatzantoni, K.; Biagioli, T.; Lolli, F.; Deraos, S.; Papathanasopoulos, P.; et al. Design And Synthesis of a Novel Potent Myelin Basic Protein Epitope 87–99 Cyclic Analogue: Enhanced Stability and Biological Properties of Mimics Render

- Them a Potentially New Class of Immunomodulators†. *J. Med. Chem.* **2005**, *48*, 1470–1480, doi:10.1021/jm040849g.
17. Matsoukas, J.; Apostolopoulos, V.; Mavromoustakos, T. Designing peptide mimetics for the treatment of multiple sclerosis. *Mini-Reviews Med. Chem.* **2001**, *1*, 273–282, doi:10.2174/1389557013406873.
  18. Tapeinou, A.; Androutsou, M.-E.; Kyrtata, K.; Vlamis-Gardikas, A.; Apostolopoulos, V.; Matsoukas, J.; Tselios, T.; Vlamis, A. Conjugation of a peptide to mannan and its confirmation by tricine sodium dodecyl sulfate–polyacrylamide gel electrophoresis. *Anal. Biochem.* **2015**, *485*, 43–45, doi:10.1016/j.ab.2015.06.010.
  19. Tselios, T.; Apostolopoulos, V.; Daliani, I.; Deraos, S.; Grdadolnik, S.G.; Mavromoustakos, T.; Melachrinou, M.; Thymianou, S.; Probert, L.; Mouzaki, A.; et al. Antagonistic Effects of Human Cyclic MBP87–99 Altered Peptide Ligands in Experimental Allergic Encephalomyelitis and Human T-Cell Proliferation. *J. Med. Chem.* **2002**, *45*, 275–283, doi:10.1021/jm0102147.
  20. Tselios, T.V.; Lamari, F.; Karathanasopoulou, I.; Katsara, M.; Apostolopoulos, V.; Pietersz, G.A.; Matsoukas, J.M.; Karamanos, N.K. Synthesis and study of the electrophoretic behavior of mannan conjugates with cyclic peptide analogue of myelin basic protein using lysine-glycine linker. *Anal. Biochem.* **2005**, *347*, 121–128, doi:10.1016/j.ab.2005.09.014.
  21. Tzakos, A.; Kursula, P.; Troganis, A.; Theodorou, V.; Tselios, T.; Svarnas, C.; Matsoukas, J.; Apostolopoulos, V.; Gerothanassis, I. Structure and Function of the Myelin Proteins: Current Status and Perspectives in Relation to Multiple Sclerosis. *Curr. Med. Chem.* **2005**, *12*, 1569–1587, doi:10.2174/0929867054039026.
  22. Deraos, G.; Chatzantoni, K.; Matsoukas, M.-T.; Tselios, T.; Deraos, S.; Katsara, M.; Papathanasopoulos, P.; Vynios, D.; Apostolopoulos, V.; Mouzaki, A.; et al. Citrullination of Linear and Cyclic Altered Peptide Ligands from Myelin Basic Protein (MBP87–99) Epitope Elicits a Th1 Polarized Response by T Cells Isolated from Multiple Sclerosis Patients: Implications in Triggering Disease. *J. Med. Chem.* **2008**, *51*, 7834–7842, doi:10.1021/jm800891n.
  23. Katsara, M.; Deraos, G.; Tselios, T.; Matsoukas, J.; Apostolopoulos, V. Design of Novel Cyclic Altered Peptide Ligands of Myelin Basic Protein MBP83–99 That Modulate Immune Responses in SJL/J Mice. *J. Med. Chem.* **2008**, *51*, 3971–3978, doi:10.1021/jm8000554.
  24. Katsara, M.; Matsoukas, J.; Deraos, G.; Apostolopoulos, V. Towards immunotherapeutic drugs and vaccines against multiple sclerosis. *Acta Biochim. et Biophys. Sin.* **2008**, *40*, 636–642, doi:10.1111/j.1745-7270.2008.00444.x.
  25. Matsoukas, J.; Apostolopoulos, V.; Lazoura, E.; Deraos, G.; Matsoukas, M.-T.; Katsara, M.; Tselios, T.; Deraos, S. Round and round we go: Cyclic peptides in disease. *Curr. Med. Chem.* **2006**, *13*, 2221–2232, doi:10.2174/092986706777935113.
  26. Katsara, M.; Yuriev, E.; Ramsland, P.A.; Deraos, G.; Tselios, T.; Matsoukas, J.; Apostolopoulos, V. A double mutation of MBP83–99 peptide induces IL-4 responses and antagonizes IFN- $\gamma$  responses. *J. Neuroimmunol.* **2008**, *200*, 77–89, doi:10.1016/j.jneuroim.2008.06.013.
  27. Lourbopoulos, A.; Deraos, G.; Matsoukas, M.-T.; Touloumi, O.; Giannakopoulou, A.; Kalbacher, H.; Grigoriadis, N.; Apostolopoulos, V.; Matsoukas, J. Cyclic MOG 35 – 55 ameliorates clinical and neuropathological features of experimental autoimmune encephalomyelitis. *Bioorganic Med. Chem.* **2017**, *25*, 4163–4174, doi:10.1016/j.bmc.2017.06.005.
  28. Lourbopoulos, A.; Matsoukas, M.-T.; Katsara, M.; Deraos, G.; Giannakopoulou, A.; Lagoudaki, R.; Grigoriadis, N.; Matsoukas, J.; Apostolopoulos, V. Cyclization of PLP139–151 peptide reduces its encephalitogenic potential in experimental autoimmune encephalomyelitis. *Bioorganic Med. Chem.* **2018**, *26*, 2221–2228, doi:10.1016/j.bmc.2017.12.024.
  29. Apostolopoulos, V.; Barnes, N.; Pietersz, G.A.; McKenzie, I.F. Ex vivo targeting of the macrophage mannose receptor generates anti-tumor CTL responses. *Vaccine* **2000**, *18*, 3174–3184, doi:10.1016/s0264-410x(00)00090-6.
  30. Apostolopoulos, V.; Pietersz, G.A.; Gordon, S.; Martínez-Pomares, L.; McKenzie, I.F. Aldehyde-mannan antigen complexes target the MHC class I antigen-presentation pathway. *Eur. J. Immunol.* **2000**, *30*, 1714–1723.
  31. Apostolopoulos, V.; Pietersz, G.A.; Loveland, B.E.; Sandrin, M.S.; McKenzie, I.F. Oxidative/reductive conjugation of mannan to antigen selects for T1 or T2 immune responses. *Proc. Natl. Acad. Sci.* **1995**, *92*, 10128–10132, doi:10.1073/pnas.92.22.10128.
  32. Apostolopoulos, V. Cell-mediated immune responses to MUC1 fusion protein coupled to mannan. *Vaccine* **1996**, *14*, 930–938, doi:10.1016/0264-410x(95)00258-3.

33. Apostolopoulos, V.; A Pietersz, G.; Tsibanis, A.; Tsikkinis, A.; Drakaki, H.; Loveland, B.E.; Piddlesden, S.J.; Plebanski, M.; Pouniotis, D.S.; Alexis, M.N.; et al. Pilot phase III immunotherapy study in early-stage breast cancer patients using oxidized mannan-MUC1 [ISRCTN71711835]. *Breast Cancer Res.* **2006**, *8*, R27, doi:10.1186/bcr1505.
34. Apostolopoulos, V.; A Pietersz, G.; Tsibanis, A.; Tsikkinis, A.; Stojanovska, L.; McKenzie, I.F.; Vassilaros, S. Dendritic cell immunotherapy: clinical outcomes. *Clin. Transl. Immunol.* **2014**, *3*, e21, doi:10.1038/cti.2014.14.
35. Karanikas, V.; A Hwang, L.; Pearson, J.; Ong, C.S.; Apostolopoulos, V.; Vaughan, H.; Xing, P.X.; Jamieson, G.; Pietersz, G.; Tait, B.; et al. Antibody and T cell responses of patients with adenocarcinoma immunized with mannan-MUC1 fusion protein. *J. Clin. Investig.* **1997**, *100*, 2783–2792, doi:10.1172/jci119825.
36. Lofthouse, S.A.; Apostolopoulos, V.; Pietersz, G.A.; Li, W.; McKenzie, I.F. Induction of T1 (cytotoxic lymphocyte) and/or T2 (antibody) responses to a mucin-1 tumour antigen. *Vaccine* **1997**, *15*, 1586–1593, doi:10.1016/s0264-410x(97)00077-7.
37. Loveland, B.E.; Zhao, A.; White, S.; Gan, H.; Hamilton, K.; Xing, P.X.; Pietersz, G.A.; Apostolopoulos, V.; Vaughan, H.; Karanikas, V.; et al. Mannan-MUC1-Pulsed Dendritic Cell Immunotherapy: A Phase I Trial in Patients with Adenocarcinoma. *Clin. Cancer Res.* **2006**, *12*, 869–877, doi:10.1158/1078-0432.ccr-05-1574.
38. Sheng, K.-C.; Kalkanidis, M.; Pouniotis, D.S.; Esparon, S.; Tang, C.K.; Apostolopoulos, V.; A Pietersz, G. Delivery of antigen using a novel mannosylated dendrimer potentiates immunogenicity in vitro and in vivo. *Eur. J. Immunol.* **2008**, *38*, 424–436, doi:10.1002/eji.200737578.
39. Sheng, K.-C.; Kalkanidis, M.; Pouniotis, D.S.; Wright, M.D.; Pietersz, G.A.; Apostolopoulos, V. The adjuvanticity of a mannosylated antigen reveals TLR4 functionality essential for subset specialization and functional maturation of mouse dendritic cells. *J. Immunol.* **2008**, *181*, 2455–2464, doi:10.4049/jimmunol.181.4.2455.
40. Sheng, K.-C.; Pouniotis, D.S.; Wright, M.D.; Tang, C.K.; Lazoura, E.; Pietersz, G.A.; Apostolopoulos, V. Mannan derivatives induce phenotypic and functional maturation of mouse dendritic cells. *Immunol.* **2006**, *118*, 372–383, doi:10.1111/j.1365-2567.2006.02384.x.
41. Tang, C.K.; Lodding, J.; Minigo, G.; Pouniotis, D.S.; Plebanski, M.; Scholzen, A.; McKenzie, I.F.C.; Pietersz, G.A.; Apostolopoulos, V. Mannan-mediated gene delivery for cancer immunotherapy. *Immunol.* **2007**, *120*, 325–335, doi:10.1111/j.1365-2567.2006.02506.x.
42. Tang, C.-K.; Sheng, K.-C.; Pouniotis, D.S.; Esparon, S.; Son, H.-Y.; Kim, C.-W.; Pietersz, G.A.; Apostolopoulos, V. Oxidized and reduced mannan mediated MUC1 DNA immunization induce effective anti-tumor responses. *Vaccine* **2008**, *26*, 3827–3834, doi:10.1016/j.vaccine.2008.05.008.
43. Vassilaros, S.; Tsibanis, A.; Tsikkinis, A.; A Pietersz, G.; McKenzie, I.F.; Apostolopoulos, V. Up to 15-year clinical follow-up of a pilot Phase III immunotherapy study in stage II breast cancer patients using oxidized mannan-MUC1. *Immunother.* **2013**, *5*, 1177–1182, doi:10.2217/imt.13.126.
44. Apostolopoulos, V.; Lofthouse, S.A.; Popovski, V.; Chelvanayagam, G.; Sandrin, M.S.; McKenzie, I.F. Peptide mimics of a tumor antigen induce functional cytotoxic T cells. *Nat. Biotechnol.* **1998**, *16*, 276–280, doi:10.1038/nbt0398-276.
45. Apostolopoulos, V.; Osinski, C.; McKenzie, I.F. MUC1 cross-reactive Gal $\alpha$ (1,3)Gal antibodies in humans switch immune responses from cellular to humoral. *Nat. Med.* **1998**, *4*, 315–320, doi:10.1038/nm0398-315.
46. Karanikas, V.; Lodding, J.; Maino, V.C.; McKenzie, I.F. Flow cytometric measurement of intracellular cytokines detects immune responses in MUC1 immunotherapy. *Clin. Cancer Res.* **2000**, *6*, 829–837.
47. Karanikas, V.; Thynne, G.; Mitchell, P.; Ong, C.-S.; Gunawardana, D.; Blum, R.; Pearson, J.; Lodding, J.; Pietersz, G.; Broadbent, R.; et al. Mannan Mucin-1 Peptide Immunization: Influence of Cyclophosphamide and the Route of Injection. *J. Immunother.* **2001**, *24*, 172–183, doi:10.1097/00002371-200103000-00012.
48. Mitchell, P.; Quinn, M.; Grant, P.T.; Allen, D.G.; Jobling, T.W.; White, S.; Zhao, A.; Karanikas, V.; Vaughan, H.; Pietersz, G.; et al. A phase 2, single-arm study of an autologous dendritic cell treatment against mucin 1 in patients with advanced epithelial ovarian cancer. *J. Immunother. Cancer* **2014**, *2*, 16, doi:10.1186/2051-1426-2-16.
49. Dargahi, N.; Matsoukas, J.; Apostolopoulos, V. Streptococcus thermophilus ST285 Alters Pro-Inflammatory to Anti-Inflammatory Cytokine Secretion against Multiple Sclerosis Peptide in Mice. *Brain Sci.* **2020**, *10*, 126, doi:10.3390/brainsci10020126.
50. Katsara, M.; Deraos, G.; Tselios, T.; Matsoukas, M.-T.; Friligou, I.; Matsoukas, J.; Apostolopoulos, V. Design and Synthesis of a Cyclic Double Mutant Peptide (cyclo(87–99)[A91,A96]MBP87–99) Induces Altered

- Responses in Mice after Conjugation to Mannan: Implications in the Immunotherapy of Multiple Sclerosis. *J. Med. Chem.* **2009**, *52*, 214–218, doi:10.1021/jm801250v.
51. Katsara, M.; Deraos, S.; Tselios, T.; Pietersz, G.; Matsoukas, J.; Apostolopoulos, V. Immune responses of linear and cyclic PLP139-151 mutant peptides in SJL/J mice: peptides in their free state versus mannan conjugation. *Immunother.* **2014**, *6*, 709–724, doi:10.2217/imt.14.42.
52. Katsara, M.; Yuriev, E.; Ramsland, P.A.; Deraos, G.; Tselios, T.; Matsoukas, J.; Apostolopoulos, V. Mannosylation of mutated MBP83–99 peptides diverts immune responses from Th1 to Th2. *Mol. Immunol.* **2008**, *45*, 3661–3670, doi:10.1016/j.molimm.2008.04.024.
53. Katsara, M.; Yuriev, E.; Ramsland, P.A.; Tselios, T.; Deraos, G.; Lourbopoulos, A.; Grigoriadis, N.; Matsoukas, J.; Apostolopoulos, V. Altered peptide ligands of myelin basic protein (MBP87–99) conjugated to reduced mannan modulate immune responses in mice. *Immunol.* **2009**, *128*, 521–533, doi:10.1111/j.1365-2567.2009.03137.x.
54. Tseveleki, V.; Tselios, T.; Kanistras, I.; Koutsoni, O.; Karamita, M.; Vamvakas, S.-S.; Apostolopoulos, V.; Dotsika, E.; Matsoukas, J.; Lassmann, H.; et al. Mannan-conjugated myelin peptides prime non-pathogenic Th1 and Th17 cells and ameliorate experimental autoimmune encephalomyelitis. *Exp. Neurol.* **2015**, *267*, 254–267, doi:10.1016/j.expneurol.2014.10.019.



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